PRESERVATION AND INTERPRETATION OF δ³⁴S VALUES IN CHARRED ARCHAEOBOTANICAL REMAINS

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Measurement of sulfur isotope (δ³⁴S) values in charred plant remains has the potential to inform understanding of the spatial configuration and ecology of crop production. We investigated the effects of charring, manuring, oxidation and anaerobic soil conditions on modern cereal grain/pulse seed δ³⁴S values, and assessed the effect of chemical pre-treatment on charred modern and archaeobotanical grain/seed δ³⁴S values. We used these results to interpret δ³⁴S values in archaeobotanical material from Neolithic Çatalhöyük. Our results suggest that δ³⁴S values can be reliably preserved in charred grain/seeds but are subject to influence by anaerobic soil conditions, the effect depending on the timing of flooding in relation to S assimilation.

KEYWORDS: SULFUR ISOTOPES, IRMS, ARCHAEOBOTANY, NEOLITHIC, TURKEY, FARMING

Introduction
Plant sulfur isotope values (δ³⁴S) vary according to local geologic, geographic and soil conditions (Nielsen 1974 Krouse et al. 1996; Cortecci et al. 2002; Norman et al. 2006; Seal 2006; Hoefs 2008; Nehlich 2015). Direct measurement of δ³⁴S values in ancient plants could therefore inform about the landscape zone and agronomic conditions in which crops were grown, especially in conjunction with other isotopic measurements (e.g. Ferrio et al. 2005; Aguilera et al. 2008; Fiorentino et al. 2008, 2012; Heaton et al. 2009; Heier et al. 2009; Riehl et al. 2014; Bogaard et al. 2013a; Masi et al. 2014; Vaiglova et al. 2014a; Wallance et al. 2015; Styring et al. 2017). This study seeks to investigate the preservation of δ³⁴S values in charred plant remains, and to understand the causes of variability in δ³⁴S values. The objectives of this study are: 1) to test the effects of charring and manuring on δ³⁴S values of modern cereal grains, 2) to examine the effect of chemical pre-treatment on δ³⁴S values of charred modern and archaeobotanical plants, 3) to determine whether δ³⁴S values of charred ancient plant remains were reliably preserved, including experimental oxidation of modern charred plant samples using H₂O₂, 4) to determine the effects of anaerobic soil conditions on crop δ³⁴S values and 5) to apply these methods to examine archaeological material from Neolithic Çatalhöyük, central Anatolia in order to investigate ancient crop growing conditions and as a complement to previous ⁸⁷Sr/⁸⁶Sr isotope measurements (Bogaard et al. 2014).
Sulfur isotopes in plants

A variety of sulfur-containing compounds are present in soil, and are taken up by plants in the form of sulfate (SO\text{4}\textsuperscript{2-}) to perform essential growth and metabolic functions (White and Reddy 2009). Plant and soil δ\textsuperscript{34}S values vary widely depending on the rate of atmospheric deposition and weathering of sulfur-containing minerals, influence of seawater sulfate and microbial processes (Krouse et al. 1996; Seal 2006; Hoefs 2008; Nehlich 2015). Atmospheric sources typically contribute the largest proportion of plant S (Novák et al. 2001) but input from mineralization of soil organic matter must also be considered significant before modern pollution. In Europe, atmospheric deposition peaked around 1970, after which sulfur dioxide emission was reduced by legislation; deposition today is down to preindustrial levels of 100-150 years ago (Eriksen 2009). Mineralisation of organic S results in depletion in \textsuperscript{34}S by 1-5‰ relative to the organic matter (Schöenau and Bettany 1989). Aerosol and rainwater deposition of the relatively high δ\textsuperscript{34}S values of seawater sulfate (+20 ‰) can be detected inland up to 30km (Nielsen 1974; Cortecci et al. 2002; Norman et al. 2006; Böttcher et al. 2007).

Plant uptake of sulfate results in minimal isotopic fractionation (≈-1.5‰, Trust and Fry 1992; Tanz and Schmidt 2010) meaning that plant δ\textsuperscript{34}S values are closely related to the assimilated source sulfate. However, different plant parts have shown different δ\textsuperscript{34}S values: in wheat with +1.7‰ for roots, +1.3‰ for stems, +6‰ for leaves and +4.4% for grain, the soil sulphate being +3.7‰ (Tcherkez and Tea 2013). In addition to the factors described above, the bioavailable sulfate pool may be strongly influenced by isotopic fractionation in anaerobic soil conditions. Waterlogged soil diffuses oxygen poorly and as bacteria consume available oxygen within hours, the redox potential (Eh) of the soil decreases and other compounds are used as electron recipients instead (Alewell et al. 2008; Balakhnina et al. 2009; Cook et al. 2009; White and Reddy 2009; Husson 2013). Dissimilatory sulfate reduction (DSR) by sulfur-reducing organisms uses sulfate as an electron recipient for redox reactions, producing \textsuperscript{34}S-depleted sulfide (lower value δ\textsuperscript{34}S) and leaving the residual sulfate relatively \textsuperscript{34}S-enriched (higher value δ\textsuperscript{34}S) (Cook et al. 2009). These DSR processes can produce significant (-46 to -40‰) isotopic fractionation between the different soil S pools available to plants (Chambers and Trudinger 1979; Fry et al. 1982; Bottrell and Novák 1997; Mandernack et al. 2000).

Under anaerobic conditions, plants may incorporate the residual high-value δ\textsuperscript{34}S sulfate (pathway B2, Fig. 1). However, the reduced low-value δ\textsuperscript{34}S sulfide still remains in the soil and can also be absorbed by plants as sulfide or it can be re-oxidized and incorporated as sulfate. Sulfur uptake from either of these isotopically different sources will depend on a plants’ ability to transport oxygen to the root system or to tolerate sulfide toxicity (Fry 1986; Trust and Fry 1992; Spence et al. 2001; Finlay and Kendall 2007). As the re-oxidation of sulfide results in minimal isotopic fractionation (Chambers and Trudinger 1979), the sulfate produced by the re-oxidation of sulfide (pathway B3, Fig. 1) will have much lower δ\textsuperscript{34}S values than residual sulfate leftover from DSR (Alewell and Gehre 1999; Mößig et al. 1999; Groscheová et al. 2000; Mandernack et al. 2000; Papadimitriou et al. 2006). This situation becomes further complicated when aerobic conditions return (scenario C, Fig. 1) because the re-oxidized DSR products (with significantly lower δ\textsuperscript{34}S values) are made available for normal assimilation (pathway C3).
The seasonal effect of aerobic/anaerobic conditions results in changes of 4-11‰ in soil sulfate δ^{34}S values, with primarily high-value δ^{34}S SO_{4}^{2-} during wet periods (i.e. largely B2) and low-value δ^{34}S SO_{4}^{2-} in dry periods, derived from 34S-depleted sulfide formed by DSR during wet months (C3) (Mörth et al. 1999; Mandernack et al. 2000; Eimers et al. 2004; Otero et al. 2008; Björkvald et al. 2009). Consequently, predicting “flooded” plant δ^{34}S values relative to those of plants not subject to aerobic/anaerobic cycles is complex since plant δ^{34}S values will depend on the timing of plant growth and sulfur assimilation in relation to this cycling.

Preservation of S and δ^{34}S values in archaeological charred grain and seeds
Previous experimental work suggests that the charring process preserves a significant component of the original carbon and nitrogen, with minimal isotopic offset (Fraser et al. 2013; Styring et al. 2013; Nitsch et al. 2015). As is the case for nitrogen, a large amount of grain/seed sulfur exists as amino acids (cysteine and methionine), which when charred undergo Maillard reactions with plant starches to produce complex organic molecules; these molecules protect the nitrogen from microbial attack over time (Knicker et al. 1996; Silván et al. 2006; Styring et al. 2013). Since sulfur from cysteine and methionine, and other S-containing compounds, can also become bound in the high-molecular weight products of Maillard reactions, it is also likely to preserve archaeologically.

The potential for biochemical alteration of original sulfur-containing molecules in ancient grains has not been investigated and is an important issue in our study. However, the preservation of organic material, especially charred organic material (“biochar”) has been widely studied and provides background for our new work (Cohen-Ofri et al. 2006, 2007; Zimmerman 2010). These studies have shown that organic material can degrade due to biotic (microbial) or abiotic (e.g. chemical oxidation) pathways. Abiotic oxidation is the dominant mode of oxidation in the early stages of degradation of carbon-based biochars (Cheng et al. 2006). The exposed surfaces of biochar gain more O-containing functional groups (e.g. carboxylic acid) and become more hydrophilic over time in the presence of chemical oxidants (Moreno-Castilla et al. 2000), ozone (Kawamoto et al. 2005), and oxygen in air (Cheng et al. 2006; Cohen-Ofri et al. 2006, 2007). Experimental degradation of organic material has employed bacterial inoculation (aerobic and anaerobic) and progressive oxidation with H_{2}O_{2} (Charrié-Duhaut et al. 2000; Lehmann et al. 2005; Zimmerman 2010; Olivier 2011).

Organic S in bitumens, especially low-molecular weight compounds (Charrié-Duhaut et al. 2000), is lost over time, due to a combination of water evaporation and also biotic/abiotic oxidation (Fedorak and Grbić-Galić 1991; Ohshiro and Izumi 1999). Oxidative cleavage releases low-molecular weight subunits (including sulfones) which are more soluble in water and may be completely removed while also transforming the non-soluble portion of petroleum into more soluble forms similar to humic acids Charrié-Duhaut et al. 2000).

Methods
Test 1: Effect of charring and manuring on δ^{34}S values
Samples of ripe bread wheat grains (T. aestivum, L.), manured and unmanured, were taken from three experimental stations: Askov, Denmark; Rothamsted, UK; and Bad
Lauchstädt, Germany (Körschens and Pfefferkorn 1998; Christensen et al. 2006; Rothamsted Research 2006). At Askov and Rothamsted (Broadbalk Winter Wheat) samples derived from experimental plots that were either fertilized with farmyard manure (“FYM”), or unmanured (“NIL”), while at Bad Lauchstädt (Static Fertilization Experiment) manured samples came from plots receiving a lower or higher rate of manuring. Details of the growing conditions are reported in Fraser et al. (2011). Random samples of grains were taken from each plot and divided into three aliquots of ~ 50 grains. One aliquot from each plot was left uncharred, while the other two were charred at 230°C for six and 24 hours respectively, in a pre-heated Gallenkamp (London, UK) Plus II electric oven, following the procedure described by Nitsch et al. (2015).

**Test 2: Effect of pre-treatment on δ34S values in charred modern and archaeobotanical material**

Laboratory pre-treatment of archaeobotanical remains for radiocarbon dating routinely employs an Acid-Base-Acid (ABA) chemical pre-treatment to remove exogenous carbon. While Vaiglova et al. (2014b) recently investigated the effectiveness of pre-treatment methods for plant δ13C and δ15N analysis, the effect of ABA treatment on δ34S is unknown and must be investigated since, in future, samples available for destructive analyses such as δ34S measurement may have already been subject to ABA treatment. Charred grain (bread wheat, barley and cereal indeterminate) and pulses (bitter vetch) from medieval (UK) and Roman (UK) contexts was measured to assess the effect of ABA pre-treatment. While the degree of contamination in this archaeobotanical material is unknown, these results were compared to two (uncontaminated) modern charred einkorn samples in order to assess chemical alteration due to pre-treatment. Prior to pre-treatment, each collection of charred grains/seeds was divided into two groups, one of which was subjected to a gentle Acid-Base-Acid pretreatment (identical to “ABA-neutrality” method reported by Vaiglova et al. 2014b). Samples were treated with 1M HCl (aq.) at 80°C for 30 minutes (until effervescence ceased), then 0.1M NaOH (aq.) for 25 minutes, before repeating the first acid stage. Samples were rinsed 3x with Milli-Q water (Merck Millipore, division of Merck KGaA, Darmstadt, Germany) between stages. The remaining group was left untreated for comparison.

**Test 3: Experimental oxidation of modern einkorn and lentils**

Three different experimental oxidation experiments were undertaken (Table 1) under a range of different solution strengths and heating conditions, designed to simulate a progression of oxidation from none (water control) to complete (30‰ (w/w) H2O2 at 80°C for 1 week). A group of ~1000 grains/seeds of organic lentils (Lens culinaris, L.), einkorn (Triticum monococcum, L., both purchased from Aroma Plantes, Sault, France), and faro/emmer (Triticum dicoccum, Bontà della Garfagnana, Italy) were charred for 24h at 230°C. The charred grains were subdivided into aliquots weighing ~ 1.0g (~30 seeds/grains) and transferred to glass tubes with loose screw cap lids for treatment with 50mL H2O2 (for details see Table 1). Heat-treated samples were heated to 80°C using a hot block.

**Test 4: Variability in δ34S values due to anaerobic soil conditions**

Test 4A) Irrigated and flooded rice, Spain:
Rice grown in aerobic soil conditions (rain-fed supplemented with irrigation) had significantly lower concentrations of arsenic (As) compared to adjacent plots grown
under conventional flooded conditions, thought to be due to the low Eh potential of
the flooded soils allowing greater mobilization of As (Moreno-Jiménez et al. 2014).
This study analysed δ34S values of the same rice aliquots measured by Moreno-
Jiménez et al. (2014) to determine the relationship between soil Eh potential and rice
δ34S values, given rice’s rhizosphere adaptations to anaerobic conditions. This
consisted of three replicate plots of rice grown under a) conventional flooded
cultivation, b) the first year of a switch to rain-fed plus sprinkler irrigation, c) the
seventh year of the continued rain-fed plus sprinkler regime.

Test 4B) Wild plants, Israel:
Hartman and Danin (2010) recently demonstrated a positive relationship between
increasing aridity and δ15N values in wild plants collected across a large rainfall
gradient in Israel, and noted that plants growing in the streambeds of seasonally
flooded wadis had higher δ15N than plants growing on adjacent exposed ridges. Here,
we measured δ34S values of the same aliquots from plants growing in wadis and
exposed ridges at four of the locations studied by Hartman and Danin (2010). All
plants were collected during the 2007 wet season and included a variety of different
plant types from each location (shrubs, dwarf shrubs, C4 dwarf shrubs, forbs,
geophytes). Different locations do not correspond to differences between plant types.

Test 4C) Cereals and wild grasses Konya plain, Turkey:
Modern cultivated cereal and wild grass species were collected from the Konya plain
in central Turkey in summer, 2011 (Fig. 2). The typical waterlogging conditions at
each sampling location were assessed by observation of the terrain and interviews
with local inhabitants. Plants that were waterlogged at the time of collection were
distinguished from those in dry locations that were not prone to flooding. The soil
substrate was identified using local maps (de Meester 1970; Bogaard et al. 2014).
Several whole ripe plants were taken from each location, and uncharred cereal/grass
grains from multiple plants were analysed.

Test 5: Measurement of δ34S values in archaeobotanical samples
Twenty charred archaeobotanical samples of a variety of taxa from Neolithic
Çatalhöyük, in the southern Konya plain, central Anatolia (c. 7100-6000 cal BC)
(Bayliss et al. 2015), were measured for δ34S and δ15N values. Different plant parts
were analysed depending on the species: cereal grains, lentil seeds, reed culm/stem,
nut shell and nut meat. All samples had been previously treated using an ABA
protocol identical to Test 2.

Analytical methods
Fresh modern seeds/grains were threshed/dehulled and homogenized using a SPEX
(Stanmore, UK) 6850 FreezerMill prior to analysis. Samples that were chemically
treated were freeze-dried before homogenization. Charred samples (archaeological
and modern) were homogenized in an agate mortar and pestle prior to analysis.

Aliquots from each sample were weighed into tin capsules for analysis with ~2mg
V2O5 to aid combustion for measurement of δ34S values. Analysis of sulfur and
nitrogen isotopes was by Continuous Flow Isotope Ratio Mass Spectrometry
(CFIRMS). The instrumentation is comprised of an Elemental analyzer (Flash/EA,
ThermoFinnigan, Bremen, Germany) coupled to a ThermoFinnigan DeltaPlus XL
isotope ratio mass spectrometer via a ConFlo III interface (ThermoFinnigan, Bremen,
Germany). Sulfur ($\delta^{34}\text{S}$) and nitrogen ($\delta^{15}\text{N}$) isotope ratios are reported in per mil (‰) relative to the VCDT and AIR standard respectively. $\delta^{34}\text{S}$ and $\delta^{15}\text{N}$ values were calibrated using an in-house reference material BRO-2 (powdered broccoli) with expected delta values of 11.67‰ (calibrated against S-1 and S-2, IAEA) for $\delta^{34}\text{S}$ and 1.5‰ (calibrated against USGS-40 and USGS-41) for $\delta^{15}\text{N}$. For $\delta^{34}\text{S}$ values, the average 1σ reproducibility for mass spectrometry controls for these analyses was ± 0.20‰. Where samples were analysed in duplicate or triplicate, the average standard deviation was 0.24‰. For $\delta^{15}\text{N}$, the 1σ reproducibility for BRO-2 was ± 0.16‰. Statistical calculations were performed using the programming language R (3.1.3).

Results

Test 1: Effect of charring and manuring on $\delta^{34}\text{S}$ values

There are differences in $\delta^{34}\text{S}$ values between sites, as well as differences within sites related to both charring and manuring (Fig. 3, Table S1). In order to assess the effect of charring and manuring on $\delta^{34}\text{S}$ values while accounting for site-level variation, a mixed-effects linear model was used, with site as a random effect, and charring and manuring as fixed effects. All levels of manuring were grouped together as “manured”. The effect of interaction between charring and manuring was also tested, but this effect was not significant ($t[47]=-0.78, p = 0.4381$), and therefore the model without the interaction effect was used because it resulted in a more parsimonious fit (AIC = 95.33 for interaction model, 93.35 for simple model). Although the manured wheat grains had $\delta^{34}\text{S}$ values significantly lower than unmanured grains (0.4‰, $p = 0.0121$), the difference was very small, and similar to the reported analytical uncertainty. There was also a small but significant effect for charring, with charred samples having lower $\delta^{34}\text{S}$ values than uncharred samples (estimated difference = 0.7‰, $p < 0.0001$).

As expected due to loss of free and chemically-bound water (Styring et al. 2013), the charred samples have higher %S compared to the uncharred samples (0.13 ± 0.02% vs. 0.09 ± 0.01%, Table S1). There was no difference in %S content between manured and unmanured samples.

Test 2: Effect of pre-treatment on $\delta^{34}\text{S}$ values in charred modern and archaeobotanical material

ABA-treatment did not have a measurable effect on the %S content of samples given typical analytical precision (Fig. 4a, Table S2). In both modern charred and archaeobotanical material, $\delta^{34}\text{S}$ values were consistently lower for ABA-treated samples compared to untreated replicates, between -0.4 to -3.1‰, averaging -1.9‰ (Fig. 4b) (multiple linear regression testing for effect of the individual sample and treatment: $\beta = -1.917$, $t=-4.304$, $p = 0.005$, $R^2 = 0.87$). %S was considerably lower in ancient charred samples (of various age and geographic origin) compared to modern charred grain (Fig. 4a; Table S2; see also Test 5). There were no differences in %S between charred archaeological cereal grains (barley, bread wheat) and pulses (lentils, vetches) from the same site.

Test 3: Experimental oxidation of modern charred einkorn and lentils

Experimental oxidation of progressively increasing strength (from 0.1% H$_2$O$_2$ 20°C, for 1 week, then 1% H$_2$O$_2$ at 80°C for 1-8 weeks and finally 30% H$_2$O$_2$ at 80°C for 1 week) results in a gradually decreasing %S (see Fig. 5a-c; Table S3). The samples that received the harshest treatment had significant loss of S, and the morphology of
the grains/seeds was severely altered, leaving only an orange residue. Those that received gentle treatment at room temperature did not exhibit any significant loss of S (see Fig. 5b). Samples treated with relatively dilute (1%) H2O2 for between 1-8 weeks showed up to 50-60% loss of %S, which is similar to the %S observed in archaeological samples, while the original morphology of the grain for these samples was also preserved. There is no significant relationship between δ34S values and %S (multiple linear regression testing for effect of species and %S, for %S: β = -3.2072, t=-0.397, p = 0.69, R2 = 0.91, Fig. 8), even when species are considered separately (see Fig. 5a and 5c).

The average difference between the four long-term experiments that included treatment with relatively dilute (1%) H2O2 (1-8 weeks), which most closely resembled the archaeological samples, and the water-treated control for both lentil and einkorn, was 0.6‰ (multiple linear regression testing for effect of species and treatment type, for treatment type: β = 0.55, t=-1.449, p = 0.185, R2 = 0.8422), a difference which is not significant (95% CI[-0.2, 1.3‰]) based on the small number of replicates.

Test 4: Variability in δ34S values due to anaerobic soil conditions
4A) Irrigated and flooded rice, Spain:
Flooded (conventionally grown) rice from Spain had similar δ34S values to rice that had been grown in fields sprinkler-irrigated for 7 years (9.1 ± 0.6‰ for flooded, 9.8 ± 0.8‰ for 7-year Table S4, Fig. 6). Rice harvested after the first year of the transition to sprinkler-irrigation (from flooding) had lower δ34S values (7.7 ± 1.5‰), but differences between treatment groups were not significant (F[2,6] = 3.18, p = 0.11). The aerobic/anaerobic status was confirmed by the Eh potential of the soil, which changed dramatically between flooded conditions (19±2 mV) and the first year of irrigation (457±14 mV), but remained consistent (429 ± 2 mV) once the irrigation conditions were established (Moreno-Jiménez et al. 2014).

4B) Dry wadi and exposed ridge plants, Israel:
Plants growing at the bottom of dry wadis had higher δ34S values than those grown on exposed ridges (Fig. 7a, Table S5), by an average of 2.1‰ (t=-3.14, p = 0.004) although there was a notably wide range of δ34S values in exposed ridge samples from location 2 (Ezuz). Plants from wadis had significantly higher δ15N values than plants from exposed ridges (Hartman and Danin 2010) but in the subset of samples analysed here, this difference was not quite significant for δ15N values, and accordingly the overall correlation between δ34S and δ15N values, which is positive at 3 out of the 4 sampling locations (Fig. 7b), was also not significant (R2 = 0.018, p = 0.35, for 3 out of 4 sampling locations).

4C) Konya plain, Turkey:
Modern plant samples from the Konya plain were separated by landscape zone, distinguishing between soil substrate and local waterlogging conditions (Fig. 8, Table S6). The results from wild grasses and domesticated cereal species were considered together, since each taxon was sampled from a variety of landscape zones. Samples taken from flooded or habitually flooded contexts (in alluvial and marl/sandplain soil substrates) had significantly lower δ34S values (< 3.5‰) compared to samples from non-flooded conditions, with samples from dry alluvial contexts having notably higher δ34S values (6.5 to 8.0‰). Unlike Israel, plants associated with flooded conditions had significantly lower δ34S values compared to non-flooded comparators.
Nitrogen isotope measurements of plants from flooded and non-flooded areas are highly variable, between 3.1 and 9.2‰ in flooded areas and between -2.7 and 9‰ for δ¹⁵N values in non-flooded areas. There was no correlation between nitrogen and sulfur isotope values.

**Test 5: Measurement of δ³⁴S values in archaeobotanical samples**

The results presented above suggest that charring decreases δ³⁴S values by ~ 0.7‰ (standard error 0.1467), while ABA-pretreatment decreases δ³⁴S values by 0.4 to 3.1‰ (on average 1.9‰, standard error 0.4456), and the average offset due to oxidation was 0.6‰ (standard error 0.34). Future experimental work would be able to refine the estimates of offsets due to charring or oxidation, while effects due to ABA-pretreatment could be avoided. The Çatalhöyük samples for test 5 were treated using ABA for previous research, and so δ³⁴S values can only be compared directly by adjusting for these effects. Considering all of the potential offsets, measured δ³⁴S values may need to be adjusted by +3.2±0.6‰ so that they could be compared with the results obtained from modern samples in the Konya plain presented above (Fig. 9).

The majority of the archaeological material (Table S7) had very consistent δ³⁴S values between 3 and 5‰, which – when adjusted to between 6.2 and 8.2‰ – are similar to the modern plant samples grown in dry alluvial conditions (Test 4C) in marls/sandplain and limestone landscape zones. While the majority of the adjusted archaeological δ³⁴S values were similar to alluvial contexts unaffected by flooding, some (2/17) of the measured values were significantly lower than 3‰ (unadjusted) and, even when accounting for possible effects of charring and pre-treatment, were similar to values measured in modern plants sampled from contexts that were prone to persistent waterlogging. The two samples with very low δ³⁴S values were both wheats (wheat indeterminate and einkorn wheat).

**Discussion and conclusions**

The key implications of our results are as follows (see Supplementary Information online for more extended discussion of these points).

1) Charring has a small but predictable effect on plant δ³⁴S values (-0.7‰).

2) δ³⁴S values in ABA-treated samples are consistently lower than untreated samples. Gentle ABA treatment is likely to alter δ³⁴S values to a greater extent than any potential unremoved contaminant would, similar to effects noted for pre-treatment and δ¹⁵N values (Zimmerman 2010). We recommend limited use of chemical pretreatment for δ³⁴S analysis of charred archaeological plant material.

3) Unlike N, S in charred archaeological samples is ½ to ⅓ as abundant when compared to modern charred samples. While this may reflect inherent differences between ancient and modern cultivars, it may also reflect loss of S through microbiologically or chemically mediated oxidation. Progressive oxidation of modern charred samples with different strengths of H₂O₂ at different temperatures results in %S loss that is comparable with archaeological samples. Chemical oxidation decreased δ³⁴S values, but this effect is minimal (0.6‰) for samples that underwent moderate, prolonged oxidation, which were the closest analogy for archaeological
material. This difference was not statistically significant for the small number of samples reported here, but this effect deserves further consideration for the precise application of δ³⁴S measurements.

4) In soil, several processes occur concurrently and the isotopic fractionation associated with a particular transformation can be confounded with other effects. Thus, the relationship between anaerobic soil conditions and plant δ³⁴S values is complex and it is difficult to disentangle differences in the source soil S due to atmospheric input and the potential effects of isotopic fractionation by soil bacteria. Where non-waterlogging-adapted plants assimilate S during or immediately following flooded conditions δ³⁴S values are likely to be higher than comparators not affected by flooding (pathway B2, Fig. 1). In test 4B the extreme aridity likely restricted plant growth (and S assimilation) to the wet season where local flooding conditions persisted in wadi basins. On the other hand, where plants assimilate S only after the return of aerobic conditions, plant δ³⁴S values are likely to be lower than unaffected comparators, since plants grown in soil affected by waterlogging may assimilate the low ³⁴S products of DSR (pathway C3, Fig. 1). The plants from waterlogged areas in the Konya plain (test 4C) experienced prolonged flooding and likely restricted growth during periods of excess water, but could have assimilated the low ³⁴S products of DSR when aerobic conditions periodically returned. The potentially wide variability of plant δ³⁴S values due to localized flooding conditions may confound attempts to trace geographic origin with plant δ³⁴S values directly, but presents an opportunity to understand landscape patterns in relation to flooding in more detail.

5) Measurement of δ³⁴S values in ancient crop remains could be useful to identify cultivation in different landscape zones. At Neolithic Çatalhöyük, Turkey, the majority of plant remains sampled had a relatively narrow range of δ³⁴S values, and although the comparison with modern landscape zones depends strongly on applying precise offsets due to charring, pre-treatment and oxidation, this group of δ³⁴S results are broadly consistent with modern samples from dry alluvial, marl/sandplain or limestone soils. Two wheat samples had lower δ³⁴S values, consistent with modern samples grown in (seasonally) waterlogged soils. Overall δ³⁴S analysis corroborates other sources of evidence (Bogaard et al. 2013b, 2014; Shillito et al. 2013; Ryan 2014) for cultivation at Çatalhöyük occurring in the low-lying alluvial/marl zone adjacent to the site. Recent geoarchaeological work (Ayala et al. 2017) suggests that this was a dryland anastomising river setting with localised waterlogging; it is therefore plausible that crops were largely (if not completely) protected from such effects.

ACKNOWLEDGEMENTS
We thank Katheryn Twiss and two anonymous reviewers for helpful comments on this paper. We are grateful to Prof I. Hodder for the support of the Çatalhöyük Research Project. δ³⁴S analyses were funded by NERC Isotope Geosciences Facility Grant (grant no. IP-1322-0512). δ¹⁵N analyses were funded by the Natural Environment Research Council (NERC standard grant NE/E003761/1, PI Bogaard). We thank Rothamsted Research and the Lawes Agricultural Trust (LAT) for access to archived grain samples. The Rothamsted Long-term Experiments National Capability is supported by the UK Biotechnology and Biological Sciences Research Council (BBS/E/C/00005189) and the LAT. We also thank Bent Christensen and Ines Merbach for access to material from Askov and Bad Lauchstädt, respectively. The
Spanish rice experiment is supported by the project AGL2013-48446-C3-2-R, from the Spanish Ministry of Economy and Competitiveness. The Israel case study was supported by an NSF dissertation improvement grant (no. 0643645). A plant collection permit was granted to G. H. by the Israel Nature and Parks Authority (permit no. 2007/28558). The modern Konya plain plant collection study was partly funded by the National Science Foundation under Grant No. 0647131 (co-PIs Katheryn Twiss and Amy Bogaard).

REFERENCES


Bogaard, A., Charles, M., Livarda, A., Ergun, M., Filipovic, D., and Jones, G., 2013b, The archaeobotany of mid-later occupation levels at Neolithic Çatalhöyük, in


Masi, A., Sadori, L., Balossi Restelli, F., Baneschi, I., and Zanchetta. G., 2014, Stable carbon isotope analysis as a crop management indicator at Arslantepe (Malatya, Turkey) during the Late Chalcolithic and Early Bronze Age, *Vegetation History and Archaeobotany* 23(6), 751-60.


Styring, A.K., M. Charles, F. Fantone, M.M. Hald, A. McMahon, R.H. Meadow, G.K.


**Figure captions**

Figure 1. Potential differences in plant δ34S values due to fractionations associated with sulfate (SO$_2^-_4$) transformation during anaerobic and aerobic conditions. (A) “normal” conditions, where soil sulfate is directly assimilated by plants. When anaerobic conditions occur (B) some of the original sulfate pool is available for assimilation (B1) but this quickly becomes reduced to sulfide (e.g. H$_2$S) through
Disimilatory Sulfate Reduction (DSR). This produces reduced sulfur with extremely low δ34S (e.g. -20 ‰) values that coincide with extremely high δ34S values (e.g. +20‰) measured in the remaining sulfate. This leftover high δ34S value sulfate is available for assimilation by plants (B2), however so is low 34S sulfide, which can be re-oxidized in the rhizosphere (B3), especially of water-adapted plants. When aerobic conditions return (C) the low 34S sulfide can be re-oxidized into sulfate, which is available for assimilation (C3) without significant fractionation.

Figure 2. Map showing location of samples (numbered points) taken for δ34S analysis in the Konya plain, south-central Turkey (after de Meester 1970 and Bogaard et al. 2014: Fig. 2). The star indicates the archaeological site of Çatalhöyük.

Figure 3. δ34S values of modern bread wheat from three different growing experiments (Askov, Denmark; Bad Lauchstädt, Germany; Rothamsted, UK), comparing manured (black) and unmanured plots. Each point is an individual measurement of a homogenized powder of multiple seeds or grains, with each powder measured in duplicate or triplicate. 1σ reproducibility including all replicate samples was ± 0.24‰. Rates of manuring are 35-37 t/ha for Askov and Rothamsted, while for Bad Lauchstädt, two levels of manure are compared: FYM1 (20t/ha) and FYM2 (30t/ha). 0h = uncharred/fresh; 6h = heated at 230°C for 6 hours; 24h = heated at 230°C for 24 hours.

Figure 4. a) Comparison of %S by weight for ABA-treated and untreated charred modern cereals and pulses from modern, Medieval (UK), and Roman (UK) contexts. The average and 1σ range of modern charred cereals from test 1 is shown as a shaded region. b) δ34S values for different replicates of the same homogenized charred plant sample (cereal and pulse), comparing ABA-treated and untreated samples. 1σ reproducibility including all replicate samples was ± 0.24‰. Multiple values for Roman cereal (treated and untreated) represent multiple IRMS measurement of the same aliquot.

Figure 5. a) Differences in δ34S values for different oxidation treatments with different dilutions of H2O2 (w/w or v/v) at either room temperature or 80°C. b) %S with different oxidation treatments. c) Relationship between δ34S values and %S for chemically oxidized samples. Regression line and 95% confidence interval (shaded region) also shown. 1σ reproducibility for δ34S values including all replicate samples was ± 0.24‰.

Figure 6 Comparison of δ34S values from long-term flooded (conventional) rice, year 1 of the switch to non-flooded (sprinkler-irrigated rice) and year 7 of sprinkler-irrigated rice (Moreno-Jiménez et al. 2014). Each measurement was made of a homogenized powder of several dozen dehusked rice grains, with one measurement from each of the three replicate experimental plots. Samples from the same experimental plot are joined by lines.

Figure 7. a) Differences in δ34S values for plants growing in dry streambeds (wadis) or exposed ridges at different sites in Israel. For details of sample collection see Hartman and Danin (2010). b) Relationship between δ15N and δ34S values for plants from dry riverbeds (wadis) and exposed ridges in Israel with regression line and 95%
confidence region (shaded area) indicated. δ¹⁵N values originally published by Hartman and Danin (2010).

Figure 8. δ³⁴S values of modern plants sampled from five different landscape zones in the Konya plain, Turkey. Information about the degree of waterlogging comes from observation combined with interviews with local producers.

Figure 9. δ³⁴S values measured in archaeological samples from Neolithic Çatalhöyük, compared to δ¹⁵N values.